AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph on page 1, after the Title, which was added by Preliminary Amendment filed on November 30, 2001, as follows:

This application is a Continuation of U.S. Application Serial No. 09/448,310 filed November 24, 1999, now ~~allowed~~ US Patent No. 6,538,122, which is a Continuation of U.S. Application Serial No. 09/109,063, filed on July 2, 1998, now US Patent No. 6,013,498.

Please amend the paragraph beginning on page 6, line 18 as follows:

Namely, the present invention provides a protein having a transglutaminase activity, which comprises a sequence ranging from serine residue at the second position to proline residue at the 331st position in an amino acid sequence represented by ~~SEQ ID No. 1~~ SEQ ID NO: 1 wherein N-terminal amino acid of the protein corresponds to serine residue at the second position of ~~SEQ ID No. 1~~ SEQ ID NO: 1.

Please amend the paragraph beginning on page 6, line 24 as follows:

There is provided a protein which consists of an amino acid sequence of from serine residue at the second position to proline residue at the 331st position in an amino acid sequence of ~~SEQ ID No. 1~~ SEQ ID NO: 1.

Please amend the paragraph beginning on page 8, line 6 as follows:

The proteins having a transglutaminase activity according to the present invention comprise a sequence ranging from serine residue at the second position to proline residue at the 331st position in an amino acid sequence represented by ~~SEQ ID No. 1~~ SEQ ID NO: 1 as an essential sequence but the protein may further have an amino acid or amino acids after proline residue at the 331st position. Among these, the preferred is a protein consisting of an amino acid sequence of from serine residue at the second position to proline residue at the 331st position in an amino acid sequence of ~~SEQ ID No. 1~~ SEQ ID NO: 1.

Please amend the paragraph beginning on page 9, line 9 as follows:

Furthermore, the preferred is a DNA wherein a base sequence encoding for amino acid sequence of from sixth amino acid to ninth amino acid from the N-terminal amino acid, Thr-Pro-Pro-Ala (SEQ ID NO: 1, residues 7-10), has the following sequence.

Please amend the paragraph beginning on page 9, line 17 as follows:

Furthermore, the preferred is a DNA comprising a sequence ranging from thymine base at the fourth position to guanine base at the 993rd position in the base sequence of ~~SEQ ID NO. 2~~ SEQ ID NO: 2. In this case, more preferred is a DNA consisting of a sequence ranging from thymine base at the fourth position to guanine base at the 993rd position in the base sequence of ~~SEQ ID NO. 2~~ SEQ ID NO: 2.

Please amend the paragraph beginning on page 9, line 9 as follows:

A structural gene of MTG used for achieving the high expression is a DNA containing a sequence ranging from thymine base at the fourth position to guanine base at the 993rd position in the base sequence of ~~SEQ ID NO. 2~~ SEQ ID NO: 2. Taking the degeneration of the genetic codon, the third letter in the degenerate codon in a domain which codes for the N-terminal portion is converted to a codon rich in adenine and uracil and the remaining portion is comprised of a codon frequently used for E. coli in order to inhibit the formation of high-order structure of mRNA, though a DNA which codes for proteins having the same amino acid sequence can have various base sequences.

Please amend the paragraph beginning on page 10, line 15 as follows:

The domain ranging from the promoter to the terminator necessitated for the expression of MTG can be produced by a well-known chemical synthesis method. An example of the base sequence is shown in ~~SEQ ID NO. 3~~ SEQ ID NO: 3. In the amino acid sequence of ~~sequence No. 3~~ SEQ ID NO: 3, aspartic acid residue follows the initiation codon. However, this aspartic

acid residue is preferably removed as will be described below.

Please amend the paragraph beginning on page 13, line 8 as follows:

The protein having a transglutaminase activity is obtained in the form of a protein inclusion body and contained in the precipitates. This protein is solubilized with a denaturant or the like, the denaturant is removed and the protein is separated and purified. Examples of the denaturants usable for solubilizing the protein inclusion body produced as described above include urea (such as 8M) and guanidine hydrochloride (such as 6 M). After removing the denaturant by the dialysis or the like, the protein having a transglutaminase activity is regenerated. Solutions used for the dialysis are a phosphoric acid buffer solution, tris hydrochloride buffer solution, etc. The denaturant can be removed not only by the dialysis but also dilution, ultrafiltration or the like. The regeneration of the activity is expectable by any of these ~~technique s~~ techniques.

Please amend the paragraph beginning on page 14, line 2 as follows:

(2) The present invention provides a protein having a transglutaminase activity, which has a sequence ranging from serine residue at the second position to proline residue at the 331st position in the amino acid sequence represented in ~~SEQ ID NO. 1~~ SEQ ID NO: 1.

Please amend the paragraph beginning on page 14, line 6 as follows:

The N-terminals of MTG produced by the product transformed with recombinant DNA having a DNA represented in ~~SEQ ID NO. 3~~ SEQ ID NO: 3 was analyzed to find that most of them contained (formyl)methionine residue of the initiation codon.

Please amend the paragraph beginning on page 16, line 3 as follows:

A process for producing a protein having a transglutaminase activity, which has a sequence ranging from serine residue at the second position to proline residue at the 331st position in the amino acid sequence represented in ~~SEQ ID NO. 1~~ SEQ ID NO: 1 will be described below.

Please amend the paragraph beginning on page 16, line 7 as follows:

That is, a DNA which encodes for a protein having a transglutaminase activity and having a sequence ranging from serine residue at the second position to proline residue at the 331st position in the amino acid sequence represented in ~~SEQ ID NO. 1~~ SEQ ID NO: 1 is employed as the MTG structural gene present on recombinant DNA usable for the expression of MTG. Concretely, a DNA having a sequence ranging from thymine base at the fourth position to guanine base at the 993rd position in the base sequence of ~~SEQ ID NO. 2~~ SEQ ID NO: 2 is employed.

Please amend the paragraph beginning on page 16, line 15 as follows:

The N-terminal sequence can be altered by an ordinary DNA recombination technique, or specific site directional mutagenesis technique, a technique wherein PCR is used for the whole or partial length of MTG gene, or a technique wherein the part of the sequence to be altered is exchanged with a synthetic DNA fragment by a restriction enzyme ~~treatmen t~~ treatment.

Please amend the paragraph beginning on page 18, line 3 as follows:

As a promoter for transcripting MTG gene, trp promoter capable of easily deriving the transcription in a medium lacking tryptophan was used. Plasmid pTTG2-22 (J. P. KOKAI No. Hei 6-225775) for the high expression of transglutaminase (TG) gene of Pagrus major was obtained with trp promoter. The sequence in the upstream of the TG gene of Pagrus major was designed so that a foreign protein is highly expressed in ~~E. col i~~ E. coli.

Please amend the paragraph beginning on page 18, line 26 as follows:

The ClaI/HpaI fragment of the Synthetic DNA gene was so designed that it had EcoRI and HindIII sites at the terminal. The designed gene was divided into blocks each comprising about 40 to 50 bases so that the + chain and the - chain overlapped each other. Twelve DNA

fragments corresponding to each sequence were synthesized (~~SEQ ID Nos. 4 to 15~~ SEQ ID NOs: 4 to 15). 5' terminal of the synthetic DNA was phosphatized. Synthetic DNA fragments to be paired therewith were annealed, and they were connected with each other. After the acrylamide gel electrophoresis, the DNA fragments of an intended size was taken out and integrated in EcoRI/HindIII sites of pUC19. The sequence was confirmed and the correct one was named pUCN216. From the pUCN216, a ClaI/HpaI fragment (small) was taken out and used for the construction of pTRPMTG-01.

Please amend the paragraph beginning on page 19, line 13 as follows:

Since E. coli JM109 keeping pTRPMTG-01 did not highly express MTG, parts (777 bases) other than the N-terminal altered parts of MTG gene were altered suitably for E. coli. Since it is difficult to synthesize 777 bases at the same time, the sequence was determined, taking the frequency of using codons in E. coli into consideration, and then four blocks (B1, 2, 3 and 4) therefor, each comprising about 200 bases, were synthesized. Each block was designed so that it had EcoRI/HindIII sites at the terminal. The designed gene was divided into blocks of about 40 to 50 bases so that the + chain and the - chain overlapped each other. Ten DNA fragments of the same sequence were synthesized for each block, and thus 40 blocks were synthesized in total (~~SEQ ID Nos. 16 to 55~~ SEQ ID NOs: 16 to 55). 5' terminal of the synthetic DNA was phosphatized. Synthetic DNA fragments to be paired therewith were annealed, and they were connected with each other. After the acrylamide gel electrophoresis, DNA of an intended size was taken out and integrated in EcoRI/HindIII sites of pUC19. The base sequence of each of them was confirmed and the correct ones were named pUCB1, B2, B3 and B4. As shown in FIG. 2, B1 was connected with B2, and B3 was connected with B4. By replacing a corresponding part of pTRPMTG-01 therewith, pTRPMTG-02 was constructed. The sequence of the high expression MTG gene present on pTRPMTG-02 is shown in ~~SEQ ID No. 3~~ SEQ ID

No: 3.

Please amend the paragraph beginning on page 21, line 8 as follows:

Further, the western blotting was conducted with MTG antibody against mouse to find that MTG was expressed only slightly in the supernatant fraction obtained by the centrifugation and that the expressed MTG was substantially all in the form of insoluble protein inclusion ~~bodie s~~ bodies.

Please amend the paragraph beginning on page 23, line 24 as follows:

A base sequence corresponding to aspartic acid residue (the N-terminal of MTG) was deleted by PCR using pUCN216 containing 216 bases as the template. pUCN216 is a plasmid obtained by cloning about 216 bp's containing ClaI-HpaI fragment of N-terminal of MTG in EcoRI/HindIII site of pUC19. pF01 (~~SEQ ID No. 56~~ SEQ ID NO: 56) and pR01 (~~SEQ ID No. 57~~ SEQ ID NO: 57) are primers each having a sequence in the vector. PDELD (~~SEQ ID No. 58~~ SEQ ID NO: 58) is that obtained by deleting a base sequence corresponding to Asp residue. pHd01 (~~SEQ ID No. 59~~ SEQ ID NO: 59) is that obtained by replacing C with G not to include HindIII site. pF01 and PDELD are sense primers and pR01 and pHd01 are antisense primers.